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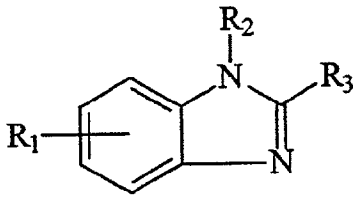
(54) METHODE DE TRAITEMENT DU CANCER ET COMPOSITIONS CONNEXES
(54) METHOD FOR TREATMENT OF CANCER AND COMPOSITIONS FOR USE THEREIN

(57)

34 The present invention provides a method for the treatment of a tumor in a subject. The method comprises administering to the subject a composition comprising a therapeutically effective amount of a compound of Formula I: <IMG> wherein R1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, -SR7, -SOR8, -SO2R9, -SCN, B'(CH2)n BR10, C(O)-R11 or -OR12, COOR13, -NO2, NR13a COOR13b , isothiocyanato, or -CN where R7 to R13b are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO2 and n is 1 to 4; R2 is selected from H, or substituted or unsubstituted alkyl. R3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, -SR14, -OR15, -SOR16, -SO2R17 -SCN, -C(O)-R18, -OR19 , NR20COOR21, where R15 to R21 are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl; or an analogue or metabolite thereof.



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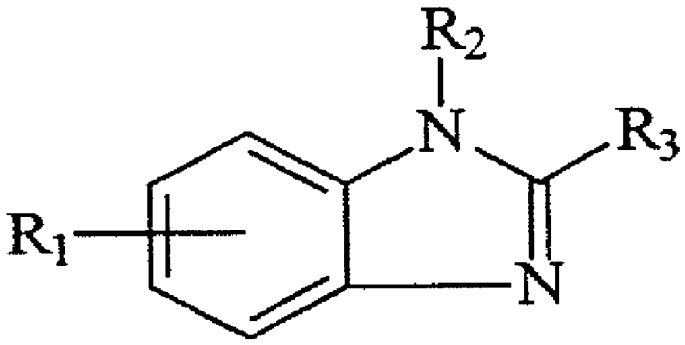
(54) Titre : METHODE DE TRAITEMENT DU CANCER ET COMPOSITIONS CONNEXES
(54) Title: METHOD FOR TREATMENT OF CANCER AND COMPOSITIONS FOR USE THEREIN



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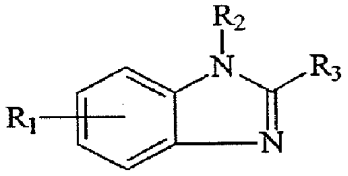
(57) Abrégé/Abstract:
The present invention provides a method for the treatment of a tumor in a subject. The method comprises administering to the subject a composition comprising a therapeutically effective amount of a compound of Formula I: (see formula I) wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_n$ BR_{10}, $C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, NR_{13a} $COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4; R_2 is selected from H, or substituted or unsubstituted alkyl. R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$ -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl; or an analogue or metabolite thereof.

ABSTRACT

The present invention provides a method for the treatment of a tumor in a subject. The method comprises administering to the subject a composition comprising a therapeutically effective amount of a compound of Formula I:



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wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;

or an analogue or metabolite thereof.

METHOD FOR TREATMENT OF CANCER AND COMPOSITIONS FOR USE THEREIN

FIELD OF THE INVENTION

The present invention is concerned with methods and compositions for the treatment of tumors.

BACKGROUND OF THE INVENTION

Hepatocellular carcinoma (HCC; hepatoma) is one of the most common malignancies and a leading cause of death worldwide (1-3). Untreated, HCC typically has a dismal prognosis. Surgical resection remains the mainstay for treatment of HCC and provides the only consistent long-term tumor-free survival (4). However, resection has been limited primarily by low resectibility rates and recurrent disease. Systemic chemotherapy as a primary treatment modality for HCC has limited value because only a small portion of patients will obtain meaningful palliation with the presently available drugs and regimens (2, 4, 5,) and because the toxicity of currently available chemotherapeutic agents often outweighs their limited benefits (6). Furthermore, liver is the most common site for metastases of colorectal carcinoma which in itself is the leading cause of cancerous death in non-smokers in the developed world (7).

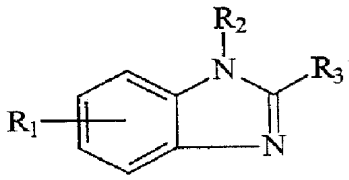
Albendazole (ABZ; methyl 5-propylthio-1H-benzimidazole-2-yl carbamate) is a benzimidazole carbamate (BZs) anthelmintic developed as a veterinary product in 1975. The BZs are now important broad-spectrum drugs for the control of helminth parasites in mammals. They are effective against lungworms and gastrointestinal nematodes, tapeworms and liver flukes (8). The intrinsic anthelmintic action of benzimidazole compounds on parasite relies on a progressive disruption of basic cell functions as a result of their binding to parasite tubulin and depolymerization of microtubules. However, a number of other mechanisms including disruption of glucose uptake and metabolism have also been described for these compounds (9-11).

SUMMARY OF THE INVENTION

The present inventors tested BZs and particularly albendazole against a range of liver (HepG2, Hep3B, PLC/PRF/5, SKHEP-1, Hep1-6, HTC, Novikoff) and colorectal cancer (C-170, HT-29 and LOVO) cell lines. The results

obtained show potent and dose dependent inhibition of proliferation of these cells by albendazole (and several other BZs). Albendazole was effective against all human and animal cell lines examined, and over a 5 day treatment period, [3H]thymidine incorporation was reduced by over 80% (range 81.6 - 99.4%) in all these cell lines.

Accordingly, in a first aspect, the present invention provides a method for the treatment of a tumor in a subject, the method comprising administering to the subject a composition comprising a therapeutically effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;

or an analogue or metabolite thereof.

DESCRIPTION OF FIGURES

Fig. 1. [^{3}H]Thymidine incorporation [expressed as counts per minute (CPM)] in SKHEP-1 cells was measured either (a) immediately after 1 day treatment with albendazole or (b) after 1 day treatment with albendazole followed by 4 days treatment with the medium alone (not containing the drug). Data points are the mean ± s.e.m.

Fig. 2. Time-course effects of albendazole on SKHEP-1 cell number. Cells growing in 6 well plates were treated for 1, 3, or 5 days with albendazole (0, 100, 500 or 1000 nM) and number of viable cells were counted using the Trypan blue exclusion method. Data points are the mean ± s.e.m.

Fig. 3. Effect of albendazole on cell cycle stage of SKHEP-1 cells. Cells were treated with different concentrations of albendazole (0, 100, 250, and 1000 nM) for 3 days, stained with propidium iodide and analyzed for DNA content by flow cytometry. A total of 10/000 nuclei were analyzed from each sample. Data points are the mean ± s.e.m. of the percentage of cells within G0-G1, S and G2-M phases of the cell cycle.

Fig. 4. Effect of different doses of albendazole (0, 50, 150 & 300 mg/kg/day in two divided dose given orally in sesame oil) on SKHEP-1 subcutaneous tumor formation and growth in nude mice. Changes in tumor volumes were measured every 3 days. Each value represents mean ± s.e.m. of 10 animals.

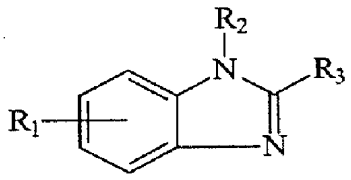
Fig. 5 Concentration dependent inhibition of 3H-thymidine uptake (proliferation) of the ovarian cancer cell line (OVCAR-3) by albendazole *in vitro*.

Fig. 6. Serum tumor marker levels (AFP or CEA) in patients with liver tumors (CRC or HCC) under treatment with albendazole (10 mg / kg / day in two or three divided oral doses) for 28 days. Arrow indicates commencement of therapy.

Fig. 7. Serum white cell count (WCC) in patients with liver tumors (CRC or HCC) under treatment with albendazole (10 mg /kg / day in two or three divided oral doses) for 28 days.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the present invention provides a method for the treatment of a tumor in a subject, the method comprising administering to the subject a composition comprising a therapeutically effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;

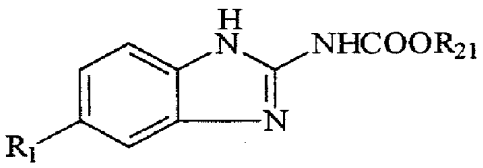
or an analogue or metabolite thereof.

Preferably R_1 substitution occurs in the 5 or 6 position and most preferably in the 5 position.

Where R_1, R_2 and/or R_3 are substituted, the substituent(s) may be independently selected from one or more of alkyl, halo, hydroxy or alkoxy.

Preferably the alkyl substituents are C_{1-6} alkyl. Preferably the aryl substituent are substituted or unsubstituted phenyl.

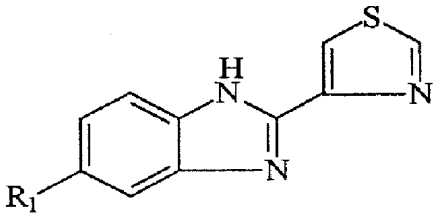
Preferably the benzimidazole compound used in the method of the present invention is benzimidazole carbamate of Formula II:



II

wherein R_1 and R_{21} are as defined above.

The compound may be a compound of Formula III

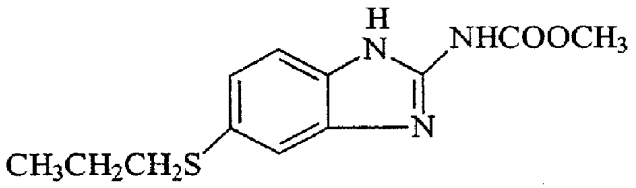


III

where R_1 is as defined above.

The compound of Formula I may be selected from albendazole, mebendazole, flubendazole, triclabendazole, oxfenbendazole, luxabendazole, cambendazole, oxibendazole, parbendazole, thiabendazole or fenbendazole.

Particularly preferred is albendazole:



or an analogue or metabolite thereof. The metabolite may be a major albendazole metabolite such as a sulphoxide or sulphone.

The method of treatment of the invention may be used to treat primary or secondary cancers. The method of the invention may be particularly suitable for the treatment of hepatoma (primary liver cancer) in a subject.

The method of the invention may also be used to treat other cancers, for example, colorectal cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer, renal cancer, sarcoma or secondary metastases thereof, particularly in the liver. In addition, the composition used in the method of the present invention may be used to treat peritoneal disease arising from ovarian, gastric or pancreatic cancers.

The method of the invention may include concomitant treatment with a potentiator of the benzimidazole compound effect on the cancer. The potentiator may be an isoquinoline compound (eg praziquantel) or any other compound which will increase or add to the effectiveness of the drug.

Albendazole is poorly absorbed from the gastrointestinal tract and also rapidly undergoes extensive first pass metabolism. At all times after the administration of a 400 mg oral dose, the concentration of the unchanged drug has been below the detection limits (18). This is mainly because of the rapid and extensive metabolism of the drug in the liver. Hydrolysis of the carbamate moiety and oxidation of the sulphur atom, the alkyl side chain and the aromatic ring have all been observed to occur in man. Five major metabolites have been identified in the human urine of which albendazole sulphoxide is the major one. The sulphoxide is biologically active and contributes to the activity of the drug. It attains peak plasma concentrations of about 200-300 ng/ml and has a plasma half life of about 8-9 hours. Together with other metabolites, it is mainly excreted in the urine with a small amount being excreted in the faeces (18,19).

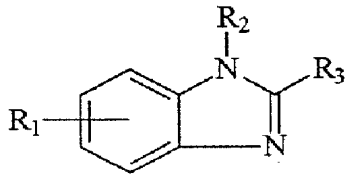
As a result of this extensive metabolism, the parent drug is virtually undetectable in the body, and its anthelmintic effect seems to be partly exerted by the unabsorbed portion left in the intestine and partly by the active sulphoxide metabolite formed in the liver. However, to be effective in the treatment of HCC a concentration of greater than 100 nM must be available in the immediate vicinity of the tumor cells which means that, to attain effective and sustained antitumor concentrations of albendazole , large and frequent doses must be administered.

Secondly, the use of the drug as an anthelmintic has been associated with a number of side effects including mild and transient epigastric distress,

diarrhoea, nausea, dizziness, lassitude and insomnia in short term treatments and reversible low grade transaminase elevation, jaundice, gastro intestinal symptoms, alopecia, rash or pruritus and leukopenia have been reported in patients under 3 month treatment courses for hydatid disease. Long term toxicity studies in animals showed diarrhoea, anaemia, hypotension, marrow depression, liver function test abnormalities, and fetal toxicity, varying by species (13).

The present inventors believe that regional administration of the benzimidazole compound to the liver may resolve the above mentioned limitations in the employment of the drug in method of treatment of the present invention. The present inventors also believe that this benefit may also be obtained through regional delivery of the benzimidazole compound to tumors of other cancers such as colorectal cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer, gastric cancer, ovarian cancer, mesothelioma or renal cancer.

Accordingly, in a second aspect the present invention consists in a method of treatment of a tumour in a subject, the method comprising regionally delivering to the site of the tumour a composition comprising a therapeutically effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

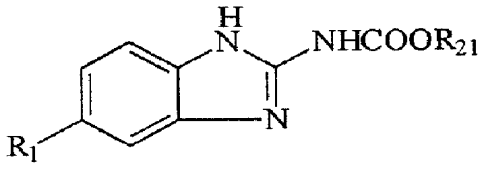
R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;
or an analogue or metabolite thereof.

Preferably R_1 substitution occurs in the 5 or 6 position and most preferably in the 5 position.

Where R_1, R_2 and/or R_3 are substituted, the substituent(s) may be independently selected from one or more of alkyl, halo, hydroxy or alkoxy.

Preferably the alkyl substituents are C_{1-6} alkyl. Preferably the aryl substituent are substituted or unsubstituted phenyl.

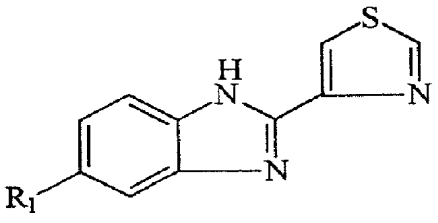
Preferably the benzimidazole compound used in the method of the present invention is benzimidazole carbamate of Formula II:



II

wherein R_1 and R_{21} are as defined above.

The compound may be a compound of Formula III

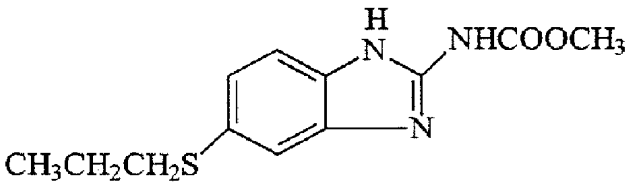


III

where R_1 is as defined above.

The compound of Formula I may be selected from albendazole, mebendazole, flubendazole, triclabendazole, oxfenbendazole, luxabendazole, cambendazole, oxibendazole, parbendazole, thiabendazole or fenbendazole.

Particularly preferred is albendazole:



or an analogue or metabolite thereof. The metabolite may be a major albendazole metabolite such as a sulphoxide or sulphone.

The method of the second aspect is particularly suitable for the treatment of tumor of the liver. The tumor may be a hepatoma (primary liver cancer) or a secondary cancer in the liver. Preferably regional delivery to the liver is via the intrahepatic artery.

The method of the second aspect of the invention may also be used to treat other cancers, for example, colorectal cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer, renal cancer or secondary metastases in other organs.

Regional delivery of the benzimidazole compound may be achieved by administering the compound in a pharmaceutically acceptable formulation. The composition may be administered as continuous infusion of a solution via a pump through the major artery of the diseased organ for example hepatic artery for hepatomas. Furthermore, the composition may be administered intraperitoneally as a suspension to treat peritoneal disease arising from ovarian, pancreatic, gastric, or any other cancer.

The formulation preferably comprises a lipid. Particularly preferred are lipids for which the tumor is avid so that high concentrations of the drug may be delivered to the tumor.

Preferably the lipid is an oil. Preferably the formulation comprises an iodised oil. A particularly preferred iodised oil is lipiodol, an iodinated ethyl ester of the poppy seed oil.

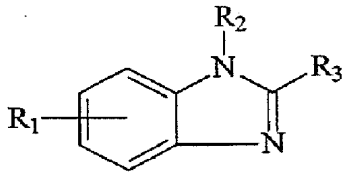
Compared to systemic administration, regional delivery using a lipid such as lipiodol allows achievement of higher drug concentrations in the tumor site while reducing the degree of exposure of other body organs to the unwanted effects of the drug and consequently reducing the number and the severity of side effects. In HCC this can be made even more selective and effective by choosing lipiodol as the vehicle for the drug delivery.

When injected into the hepatic artery, the oil is retained by HCCs for several weeks to over a year but is cleared from the normal liver parenchyma within 7 days. Without wishing to restrict the present invention in any way, one of the hypotheses in attempting to explain lipiodol retention in HCCs suggests that these cells are unable to clear lipiodol because they lack a reticuloendothelial kupffer cell component. We have previously shown that *in vitro*, vitamin D compounds such as 1, 25-dihydroxyvitamin D3 dissolved in lipiodol produce a profound and sustained inhibitory effect on HepG2 cells when injected through the hepatic artery of tumor bearing rats, the drug is retained within the tumor (See International Patent Application Nos. PCT/AU98/00440 and PCT/AU99/00323 the disclosure of which is incorporated herein by reference).

On the basis of the present inventors experience with albendazole, lipiodol, and hepatoma cell lines, they believe that administration of albendazole dissolved in an oil such as lipiodol and administered through the intrahepatic artery, will lead to the sustained release of the drug from the oil within the tumor cells leading to sustained inhibition of proliferation of the tumor cells.

These unique characteristics of lipiodol coupled with the potency and lipid solubility of albendazole, makes the combination an attractive formulation for intrahepatic arterial administration in patients with HCC.

In a third aspect, the present invention provides a pharmaceutical composition for use in the treatment of a tumour in a subject, the composition comprising a carrier and an effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;

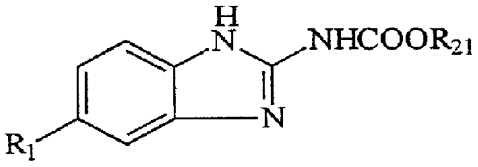
or an analogue or metabolite thereof.

Preferably R_1 substitution occurs in the 5 or 6 position and most preferably in the 5 position.

Where R_1, R_2 and/or R_3 are substituted, the substituent(s) may be independently selected from one or more of alkyl, halo, hydroxy or alkoxy.

Preferably the alkyl substituents are C_{1-6} alkyl. Preferably the aryl substituent are substituted or unsubstituted phenyl.

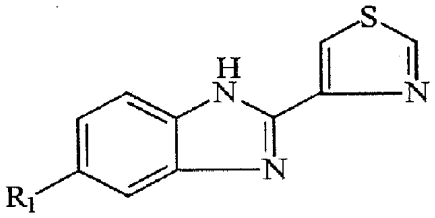
Preferably the benzimidazole compound used in the method of the present invention is benzimidazole carbamate of Formula II:



II

wherein R_1 and R_{21} are as defined above.

The compound may be a compound of Formula III

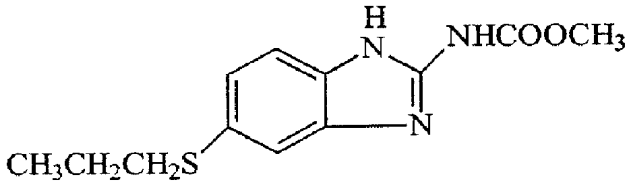


III

where R_1 is as defined above.

The compound of Formula I may be selected from albendazole, mebendazole, flubendazole, triclabendazole, oxfenbendazole, luxabendazole, cambendazole, oxibendazole, parbendazole, thiabendazole or fenbendazole.

Particularly preferred is albendazole:



or an analogue or metabolite thereof. The metabolite may be a major albendazole metabolite such as a sulphoxide or sulphone.

Preferably the carrier is a lipid. Preferably the lipid is one for which the tumor is avid. Most preferably the carrier is an oil. An iodised oil is particularly preferred. The iodised oil is preferably lipiodol.

Preferably the benzimidazole compound is present in the composition in a concentration of at least about 0.1μM. The concentration of the benzimidazole compound is preferably in the range of about 0.1 to about 10 μM.

The composition of the invention may include a potentiator of the effect of the benzimidazole compound on the cancer. The potentiator may be, for example, praziquantel or any other compound which would increase the effectiveness of the drug, have an additive effect with it, or reduce its side effects.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the nature of the present invention may be more fully understood the invention will now be described with reference to the following non-limiting embodiments.

EXAMPLE 1

IN VITRO AND *IN VIVO* SUPPRESSION OF GROWTH OF HEPATOCELLULAR CARCINOMA CELLS BY ALBENDAZOLE

Materials and Methods

Cell Culture

HepG2, Hep3-B, Hep1-6, SKHEP-1, PLC/PRF/5, and HTC cells were obtained from European Collection of Cell Cultures (ECACC; U.K), Novikoff was obtained from Cancer Research Centre (DKFZ) Heidelberg, Germany. Cells were cultured in MEM or DMEM supplemented with 10% FBS, 50 units /ml penicillin, 50 units/ml streptomycin, 25 μg/ml amphotericin B (Gibco, Grand Island, NY) and maintained subconfluent at 37° C in humidified incubators containing 5% CO_2. Albendazole (Sigma, Australian subsidiary)

was dissolved in absolute ethanol at concentrations that were 1000-fold higher than the final medium concentration.

[^{3}H]Thymidine Incorporation Assay

For the study of [^{3}H]thymidine incorporation, adherent cells (5-10 x 10^4) were plated onto 24-well Corning tissue culture dishes and were exposed to culture medium (5% FBS) containing the vehicle (0.1% ethanol) or different concentrations of albendazole (10^{-8} to 10^{-6} M). For Novikoff, a detached rat cell line, 2500 cells were suspended in 2 ml of DMEM (5%FBS) and kept under the same condition as for attached cells. Media were replaced with fresh media on alternate days. At the end of the treatment period (5 days), cell cultures were assayed for thymidine incorporation by the addition of 0.5μCi of [^{3}H]thymidine (60 Ci/mmol. ICN Biochem, Irvine, CA) to each well for the last 4 h of culture. The amount of radioactivity incorporated into cells was determined using a β-scintillation counter. Results are presented as percentage [^{3}H]thymidine incorporation relative to control. For the recovery experiments, SKHEP-1 cells were treated for 1 day with different concentrations of albendazole and then either assayed for [^{3}H]thymidine incorporation or the medium was replaced and the cells treated with fresh medium with out the drug for a further 4 days at the end of which, [^{3}H] thymidine incorporation assay was performed.

Cell Counts

SKHEP-1 cells (2.5 x 10^4) were plated in six well plates. The cell treatment procedure was as described for the thymidine assay. At the end of the treatment period (1, 3 or 5 days), cells were trypsinized and counted with a hemocytometer using the trypan blue exclusion method. In all experiments, cells treated with the medium containing 0.1% ethanol were taken as the control for albendazole treated cells. All counts were obtained in quadruplicate and each experiment was repeated at least twice.

Cell Cycle Analysis

SKHEP-1 cells (5 x 10^4) were plated onto six-well tissue culture plates. Triplicate samples were treated with the indicated concentrations of albendazole (100, 250 and 1000 nM). The medium was changed everyday. After 72 h the relevant group of cells were collected, washed twice with

phosphate buffer and treated with ribonuclease, Triton X-100 and propidium iodide (Sigma) based on the method described by Taylor [12]. The percentage of cells within the G1, S, and G2-M phases of the cell cycle were determined using a FACScan flow cytometer (Becton Dickinson FACSort) and Multifit LT cell cycle analysis software (Verity Software INC.)

Tumor formation in nude mice

6 to 10 weeks old male BALB/c Nu/Nu mice (Animal Resources Center, Perth, Australia) were inoculated subcutaneously with 10^6 SKHEP-1 cells into the right flank. 24 hours after inoculation animals were randomly assigned to one of the treatment groups (n=10 per group), receiving 25, 50 or 150 mg/kg twice daily oral albendazole suspended in sesame oil for 20 days.

The control group was treated with the vehicle (sesame oil). Using vernier calipers, tumor diameter (mm) was measured on day eight and then every three days up to day 20-post tumor cell inoculation. Tumor volumes were calculated using the formula: $ab^2/2$, where a and b are the smaller diameters in millimeters, respectively [13] and a piece of the tumor was preserved in paraffin for immunohistochemical determination of maximum proliferation index (MPI). Here, after fixation, the specimen was processed for the detection of Ki-67 antigen with the monoclonal antibody MIB1 according to the method described by McCormick [15].

The animal model was chosen on the basis of SKHEP-1 being the most tumorigenic human liver cancer cell line in nude mice [16] and previous experience with the model [17].

Statistical Analysis

Differences between different treatment groups were analyzed using ANOVA followed by Tukey's test. P values of less than 0.05 were considered to represent a significant difference.

Results

Inhibition of [^{3}H] Thymidine incorporation by albendazole

[^{3}H]Thymidine incorporation assay was used to determine the effect of albendazole on cell proliferation in a number of human (HepG2, Hep3-B, PLC/PRF/5, SKHEP-1), rat (HTC and Novikoff) and mice (Hep1-6) HCC cell lines. Results obtained show that, in all cell lines examined, albendazole

effectively reduces thymidine incorporation (Table 1). When treated with the 100 nM concentration of albendazole, compared to other cell lines, SKHEP-1 demonstrated the highest level of sensitivity to albendazole ($p<0.01$ compared to control), while the rat cell line HTC was the least responsive of all. Treatment with the 1000 nM concentration of albendazole reduced thymidine incorporation to less than 20% of control values ($p<0.001$) in all cell lines and to less than 5% in SKHEP-1 and HepG2. Here again SKHEP-1 cells displayed the highest level of sensitivity to albendazole. In these cells, thymidine incorporation was reduced to $0.6 \pm 0.1\%$ of the control values corresponding to 99.4% inhibition. For this reason, SKHEP-1 was employed for all further investigations. Exposure of SKHEP-1 cells to different concentrations of albendazole for 1 day, revealed that, concentrations of 250 nM and over of albendazole still produce profound inhibition of thymidine incorporation (Fig. 1a). Removal of the drug and treatment of cells with the normal medium for a further 4 days led to the recovery of thymidine incorporation by the cells (Fig. 1b). Except for the 500 and the 1000 nM concentrations, cells exposed to all other concentrations of albendazole were able to recover from the inhibitory effect of the drug.

Table 1: Effect of albendazole on [^{3}H] thymidine incorporation in HCC cell lines.

Cell line	[albendazole] nM			
	10	100	500	1000
HepG2	93.1 ± 5.6	72.9 ± 6.3	12.2 ± 2.9	3.5 ± 0.4
Hep3-B	105.7 ± 8.6	68.5 ± 5.7	22.6 ± 1.8	9.3 ± 0.7
SKHEP-1	89.6 ± 6.1	63.7 ± 3.1	4.5 ± 1.1	0.6 ± 0.1
PLC/PRF/5	92.7 ± 4.7	69.4 ± 5.2	26.9 ± 2.7	18.4 ± 2.1
Novikoff	96.5 ± 8.8	71.6 ± 5.9	29.2 ± 3.3	10.3 ± 1.8
HTC	98.4 ± 7.5	86.0 ± 6.9	28.5 ± 2.2	11.4 ± 1.3
Hep1-6	97.7 ± 4.3	79.5 ± 3.2	28.1 ± 2.5	5.6 ± 0.6

Cells were treated with different concentrations of albendazole (10-1000 nM) for 5 days at the end of which [^{3}H]thymidine incorporation was measured. Values (% control) represent mean ± s.e.m. of several determinations.

Albendazole inhibits proliferation of cells leading to a decline in cell number

Counting of viable cells treated with different concentrations of albendazole for 1, 3 or 5 days produced a dose dependent decline in the number of cells, showing the profound inhibition of proliferation of SKHEP-1 cells by the drug (Fig. 2).

This was evident from day 3 at the 500 and the 1000 nM concentrations. Compared to control, cells exposed to the 1000 nM concentration of the drug, were significantly reduced in number ($p<0.001$).

Dose-dependent effect of albendazole on the cell cycle kinetics

Flow cytometric analysis of albendazole-treated cells, revealed that, the drug induces a dose-dependant effect on the cell cycle kinetics of SKHEP-1 HCC cells. Fig. 3 demonstrates the changes induced on the distribution of cells in the different phases of the cell cycle following 3 days treatment with different concentrations of albendazole. From this, it is clearly evident that exposure of cells to the 250 nM concentration causes accumulation of cells in the G0-G1 phase and associated with this was a reduction in the percentage of cells in both S and G2-M phases of the cell cycle. Changes induced by the 500 nM concentration of the drug were identical to those of the 250 nM concentration (data not shown). However, as depicted in the same figure, treatment of the cells with the 1000 nM concentration of albendazole leads to a totally different pattern of changes. Here, arrest and accumulation of cells in the G2-M phase of the cell cycle was accompanied by a dramatic reduction in percentage of cells in the G0-G1 phase, while percentage of cells in the S phase remained unchanged.

Effect of albendazole on tumor growth in vivo

In control animals, SKHEP-1 tumors grew to a mean volume of 87.9 ± 12.3 mm^3 at 20 days post inoculation. In animals receiving 50 and 150 mg / kg per day, tumor growth was slightly but not significantly retarded. However, tumor growth was profoundly suppressed in animals receiving the 300 mg / kg dose of albendazole (Fig. 4) with a mean tumor volume of 12.0 ± 7.8 ($p<0.001$). Results from the immunohistochemical analysis of tumors revealed that, tumors from animals receiving the 50 and 150 mg / kg dose of albendazole had reduced MPIs of 22.54 ± 1.53 (mean ± s.e.m.) and 13.36 ± 3.04 respectively compared to 34.2 ± 3.13 for the control. There was not enough tissue for the analysis of MPI in tumors of mice receiving the 300 mg / kg / day dose.

Dose-dependent effect of albendazole on the cell cycle kinetics

Albendazole was also shown to exhibit dose dependent inhibition of proliferation of the ovarian cancer cell line (OVCAR-3) *in vitro*, (see Figure 5).

Discussion

Results from the cell proliferation studies clearly demonstrated that all human, rat and mice liver cell lines examined are profoundly inhibited by albendazole. This was manifested by the significant reduction of thymidine incorporation following treatment with albendazole doses of 100 nM and over. Similarly treatment of SKHEP-1 cells with albendazole led to a dose and time dependent reduction of cell number. The reason behind the higher sensitivity of SKHEP-1 to albendazole is not clear at this stage. However, lacking the enzymes needed for the conversion of the drug to less active or inactive metabolites, may partly account for this observation [14]. Flow cytometric analysis of the cell cycle revealed that, albendazole causes differential dose-dependent effect on the cell cycle kinetics of SKHEP-1. Accumulation of cells in the G0-G1 phase following treatment with albendazole concentrations of up to 500 nM with an associated decline in percentage of cells in S and G2-M phases, indicates that progression out of the G1 phase was blocked. Many natural triggers for programmed cell death, including glucocorticoid hormones act at G1-G0 transition and the cells die in a process described as ' premature aging' [18]. However, following treatment with the1000 nM concentration of albendazole, the pattern of cell distribution was reversed, leading to the accumulation of cells in the G2-M phase of the cycle. This indicates that the primary effect of albendazole at this concentration may be mediated by a transition delay through G2-M or mitosis.

The data from work in nude mice suggests that, at the higher dose of the 300 mg/ kg/day, albendazole presumably reaches the necessary concentrations required to suppress tumor formation. The very high rate of metabolism of albendazole in mice and the poor blood supply to the subcutaneous tumor, are amongst a number of factors that could account for the high dose of the drug required to suppress tumor growth in these animals.

The MPI data also confirm the ability of albendazole to reduce tumor proliferation rate. The Ki-67 antigen used in this assay is tightly linked to proliferation and has been used in a large number of studies to estimate the growth fraction of tumors [15].

EXAMPLE 2

ALBENDAZOLE IN PATIENTS WITH ADVANCED MALIGNANCY

Patients and Methods

The study was single-centre, open and non-controlled. Nine patients (8 male and 1 female) with either advanced CRC and hepatic metastasis or HCC were included in this study. One patient with neuroendocrine cancer and mesothelioma was also treated on a compassionate basis. The patients aged between 38-79 years were inoperable and had failed existing chemotherapy and also, except for two, had measurable and increasing tumor markers. The majority had also already failed hepatic artery chemotherapy. The diagnosis of CRC or HCC was made by ultrasound, CT or MRI scan, confirmed by histology and by determination of CEA or AFP levels for CRC or HCC respectively. Only patients with expected survival of more than one month were enrolled into the study. Patient characteristics are presented in Table 2. The study was approved by the Human Ethics Committee for Research of SESAHS. The protocol and the aim of the study was clearly explained to each patient and informed consent obtained. The duration of this study was four weeks but was to be stopped if leukopenia (WBC $< 2 \times 10^6$) or severe hepatocellular injury (ALT or AST 2 x upper limit) developed. All patients received albendazole (400 mg scored tablets, Smith Kline Beecham, Australian subsidiary) 10 mg / kg orally in two or three divided doses for a planned duration of four weeks. This is the clinical dose of albendazole employed in the treatment of parasitic diseases. Patients were evaluated every three days by clinical examination together with full blood tests to monitor tumor markers, hematopoesis, liver and kidney function toxicity. A partial response (PR) was defined as a 50% or more decrease in the value of the markers. In addition there must be no new lesions or progression of any other lesions. Stable disease was defined as a decrease of less than 50%, or an increase of less than 25% in the value of tumor markers, while progressive disease (PD) was a 25% or more increase in the value of the tumor markers or the appearance of any new lesions.

Table 2. Characteristics of the 9 patients with inoperable liver tumors, who had failed chemotherapy, participating in the phase 1 trial of albendazole.

Patient	Sex	Age	Type	Length of Treatment (days)	Comments	Site of metastatic disease
1	F	38	HCC	19	neutropenia; drug Withdrawn	MBL, lung & liver
2	M	66	CRC	14	neutropenia; drug Withdrawn	Liver & lung
3	F	62	*	28 days		Pleura & liver
4	M	66	CRC	28 days		MBL & bone
5	M	56	CRC	28 days		MBL & brain
6	M	66	CRC	28 days		Liver & lung
7	M	74	CRC	28 days		Liver & lung
8	M	54	CRC	28 days	neutropenia Withdrawn	MBL
9	M	79	CRC	28 days		MBL & peritoneum

MBL = multiple bilobar liver

* Mesothlioma and carcinoid tumor.

Results

Treatment with albendazole led to stabilization of the disease in three, and progression of the disease in the other two patients (Figure 6). In the remaining four, either the drug had to be withdrawn (2) or the tumor markers were not measurable (2). In patient number 1 suffering from HCC, albendazole treatment led to the stabilization of the disease, but because of the development of neutropenia, drug treatment was stopped in this patient on day 19. The patient visited another city after stopping albendazole and died of neutropaenic sepsis, which was almost certainly related to albendazole therapy. He had suffered neutropenia with previous chemotherapy. Patient number 2 (CRC), who was showing the greatest response to albendazole therapy, also developed neutropenia and so albendazole was also withdrawn in this patient. Patient number 3 (carcinoid tumor and mesothelioma) did not have evaluable serum tumor markers. However, the patient was monitored for adverse effects. In patients 4 and 5 there may have been a short term control of tumor markers but these began to rise again during treatment. In patients 6, 7 and 8, albendazole therapy was associated with stable CEA levels. In patient 9, the CEA levels were less than 10 μg / L and remained so for the duration of the treatment (4 weeks).

There were no significant changes in liver and kidney function tests during the course of the trial. However, in patients 1, 2 and 8 significant neutropenia developed as a result, of which the drug had to be withdrawn. WCC values for all nine patients are presented in Figure 7.

Discussion

To the present inventors' knowledge, this is the first reported study of albendazole (or any other BZ) administered to human subjects for the therapy of cancer. Patients participating in this study had advanced malignancy, which had not responded to available therapy. Administration of albendazole to the only patient in the study with primary HCC led to the stabilisation of the AFP values. However, due to neutropenia, the drug had to be withdrawn. The patient had a recent history of low WCC. In the remaining patients (all with CRC) with measurable CEA levels, three had their tumor markers stabilised while on albendazole treatment. Compared to other patients, patient number two, who was withdrawn from the trial on day 19, had the steepest fall in CEA levels.

The present report demonstrates for the first time that, albendazole, a benzimidazole carbamate with extensive clinical use as a safe antiparasitic drug, can cause tumor marker stabilisation in patients with HCC or CRC with liver metastasis.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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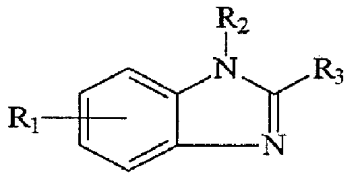
CLAIMS

The embodiments of the invention, in which an exclusive property or privilege is claimed, are defined as follows:

1. A method for the treatment of a tumor in a subject, the method comprising administering to the subject a composition comprising a therapeutically effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

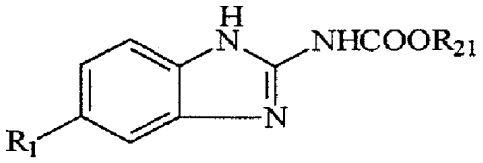
R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;

or an analogue or metabolite thereof.

2. A method as claimed in claim 1 in which the R_1 substitution occurs in the 5 or 6 position.

3. A method as claimed in claim 2 in which the R_1 substitution occurs in the 5 position.

4. A method as claimed in claim 1 in which the compound is benzimidazole carbamate of Formula II:

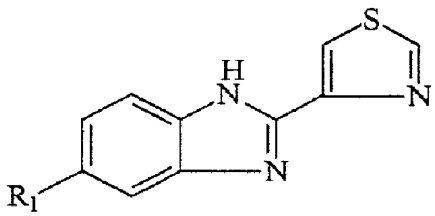


II

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_{21} is H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl.

5. A method as claimed in claim 1 in which the compound is a compound of Formula III



III

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or

unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_{21} is H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl.

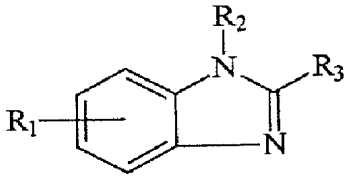
6. A method as claimed in claim 1 in which the compound is selected from the group consisting of albendazole, mebendazole, flubendazole, triclabendazole, oxfenbendazole, luxabendazole, cambendazole, oxibendazole, parbendazole, thiabendazole or fenbendazole.

7. A method as claimed in claim 1 in which the compound is albendazole or an analogue or metabolite thereof.

8. A method as claimed in claim 1 in which the tumour is hepatoma.

9. A method as claimed in claim 1 in which the tumour is selected from the group consisting of colorectal cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer, renal cancer, sarcoma and secondary metastases thereof.

10. A method of treatment of a tumour in a subject, the method comprising regionally delivering to the site of the tumour a composition comprising a therapeutically effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

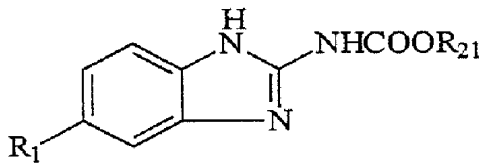
R_2 is selected from H, or substituted or unsubstituted alkyl.

R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;
or an analogue or metabolite thereof.

11. A method as claimed in claim 10 in which the R_1 substitution occurs in the 5 or 6 position.

12. A method as claimed in claim 11 in which the R_1 substitution occurs in the 5 position.

13. A method as claimed in claim 10 in which the compound is benzimidazole carbamate of Formula II:

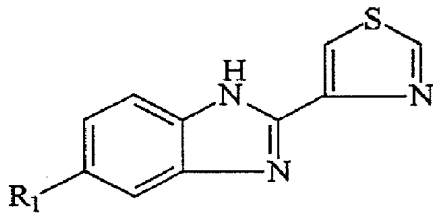


II

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_{21} is H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl.

14. A method as claimed in claim 10 in which the compound is a compound of Formula III



III

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_{21} is H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl.

15. A method as claimed in claim 10 in which the compound is selected from the group consisting of albendazole, mebendazole, flubendazole, triclabendazole, oxfenbendazole, luxabendazole, cambendazole, oxibendazole, parbendazole, thiabendazole or fenbendazole.

16. A method as claimed in claim 10 in which the compound is albendazole or an analogue or metabolite thereof.

17. A method as claimed in claim 10 in which the tumour is hepatoma.

18. A method as claimed in claim 10 in which the tumour is a secondary cancer in the liver.

19. A method as claimed in claim 17 or claim 18 in which the composition is delivered to the liver is via the intrahepatic artery.

20. A method as claimed in claim 10 in which the tumour is selected from the group consisting of colorectal cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer and renal cancer.

21. A method as claimed in claim 10 in which the composition further comprises a pharmaceutically acceptable carrier.

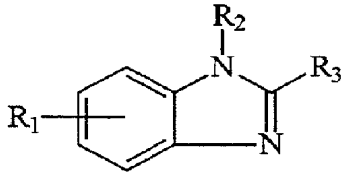
22. A method as claimed in claim 21 in which the carrier is a lipid.

23. A method as claimed in claim 22 in which the lipid is an oil.

24. A method as claimed in claim 20 in which the carrier is an iodised oil.

25. A method as claimed in claim 24 in which the iodised oil is an iodinated ethyl ester of the poppy seed oil.

26. A pharmaceutical composition for use in the treatment of a tumour in a subject, the composition comprising a lipid carrier and an effective amount of a compound of Formula I:



I

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_2 is selected from H, or substituted or unsubstituted alkyl.

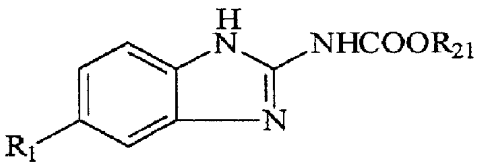
R_3 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, 5- or 6- membered heterocyclic ring the heteroatom(s) of which are selected from one of more of O, S and/or N, $-SR_{14}$, $-OR_{15}$, $-SOR_{16}$, $-SO_2R_{17}$, -SCN, $-C(O)-R_{18}$, $-OR_{19}$, $NR_{20}COOR_{21}$, where R_{15} to R_{21} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl;

or an analogue or metabolite thereof.

27. A composition as claimed in claim 26 in which the R_1 substitution occurs in the 5 or 6 position.

28. A composition as claimed in claim 27 in which the R_1 substitution occurs in the 5 position.

29. A composition as claimed in claim 26 in which the compound is benzimidazole carbamate of Formula II:

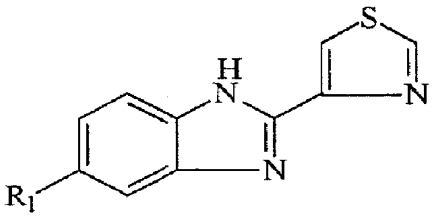


II

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, $-SR_7$, $-SOR_8$, $-SO_2R_9$, -SCN, $B'(CH_2)_nBR_{10}$, $-C(O)-R_{11}$ or $-OR_{12}$, $COOR_{13}$, $-NO_2$, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_{21} is H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl.

30. A composition as claimed in claim 26 in which the compound is a compound of Formula III



III

wherein R_1 is selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl,

cycloalkenylalkyl, aryl, arylalkyl, -SR_7, -SOR_8, -SO_2R_9, -SCN, $B'(CH_2)_nBR_{10}$, -$C(O)$-R_{11} or -OR_{12}, $COOR_{13}$, -NO_2, $NR_{13a}COOR_{13b}$, isothiocyanato, or -CN where R_7 to R_{13b} are each independently selected from H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl, arylalkyl, B and B' are independently selected from O, S, S(O) or SO_2 and n is 1 to 4;

R_{21} is H, substituted or unsubstituted, straight or branch chain alkyl, alkenyl, alkenylalkyl, cycloalkyl, cycloalkylalkyl, cycloalkenylalkyl, aryl or arylalkyl.

31. A composition as claimed in claim 26 in which the compound is selected from the group consisting of albendazole, mebendazole, flubendazole, triclabendazole, oxfenbendazole, luxabendazole, cambendazole, oxibendazole, parbendazole, thiabendazole or fenbendazole.

32. A composition as claimed in claim 26 in which the compound is albendazole or an analogue or metabolite thereof.

33. A composition as claimed in claim 26 in which the carrier is an oil.

34. A composition as claimed in claim 33 in which the carrier is an iodised oil.

35. A composition as claimed in claim 34 in which the iodised oil is an iodinated ethyl ester of the poppy seed oil.

Figure 1:

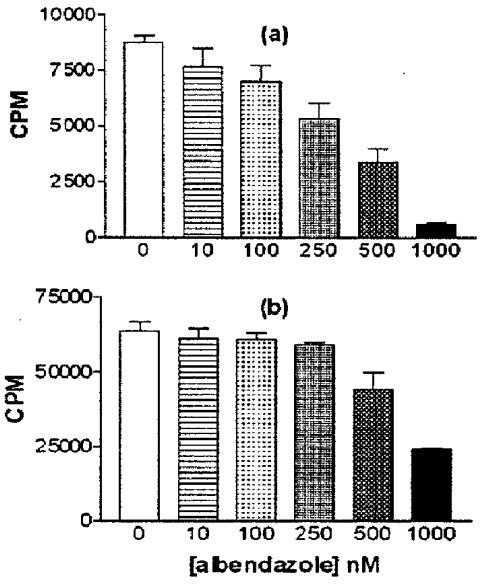

10000
7500
5000
2500
0
CPM
(a)
0
10
100
250
500
1000
75000
50000
25000
0
CPM
(b)
0
10
100
250
500
1000
[albendazole] nM

Figure 2:

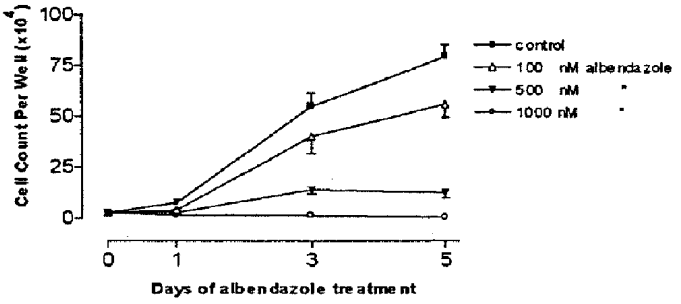

Cell Count Per Well (x10^4)
100
75
50
25
0
0
1
3
5
Days of albendazole treatment
control
100 nM albendazole
500 nM
1000 nM

Figure 3:

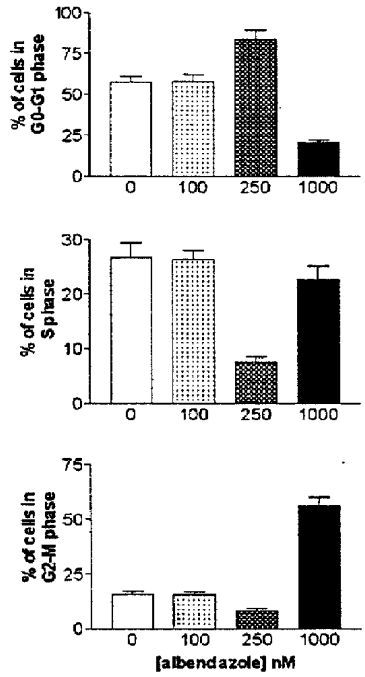

% of cells in G0-G1 phase
100
75
50
25
0
0
100
250
1000
% of cells in S phase
30
20
10
0
0
100
250
1000
% of cells in G2-M phase
75
50
25
0
0
100
250
1000
[albendazole] nM

Figure 4:

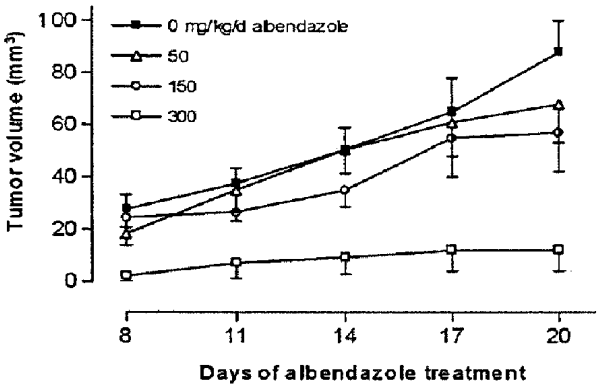

0 mg/kg/d albendazole
50
150
300
Tumor volume (mm³)
100
80
60
40
20
0
8
11
14
17
20
Days of albendazole treatment

Figure 5

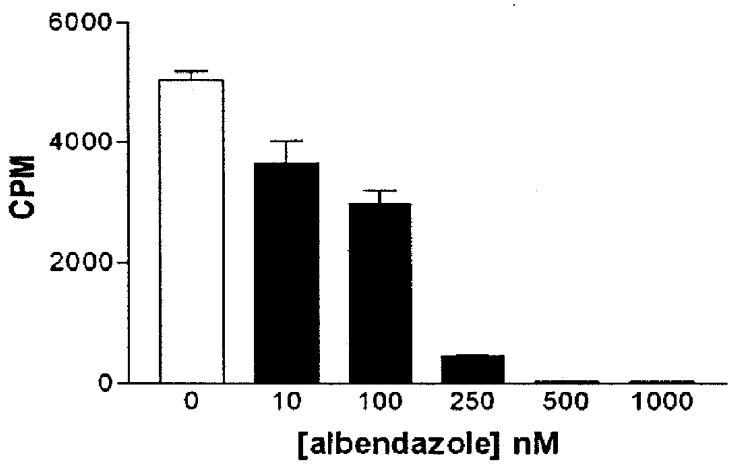


Figure 6:

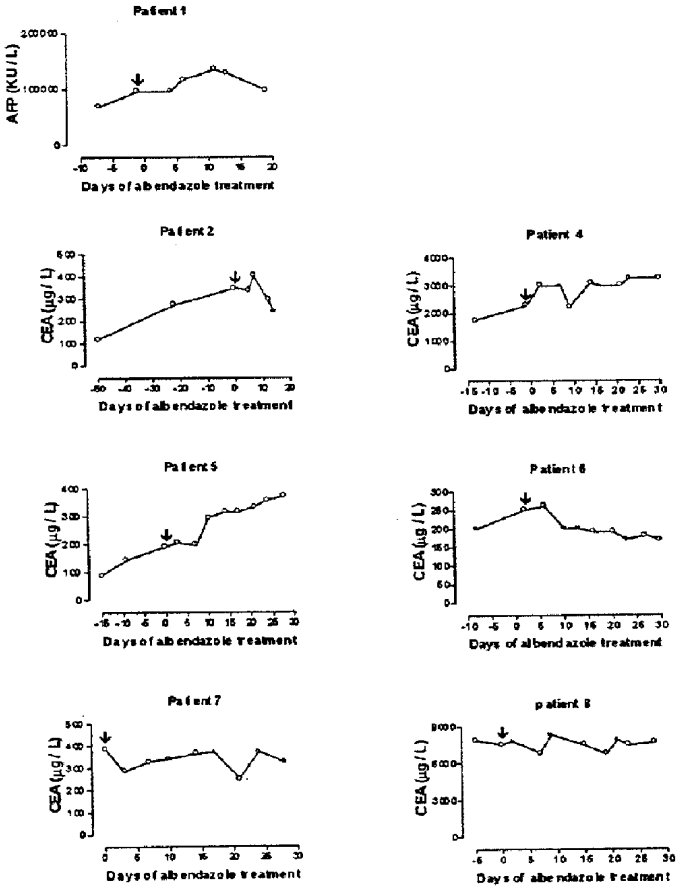
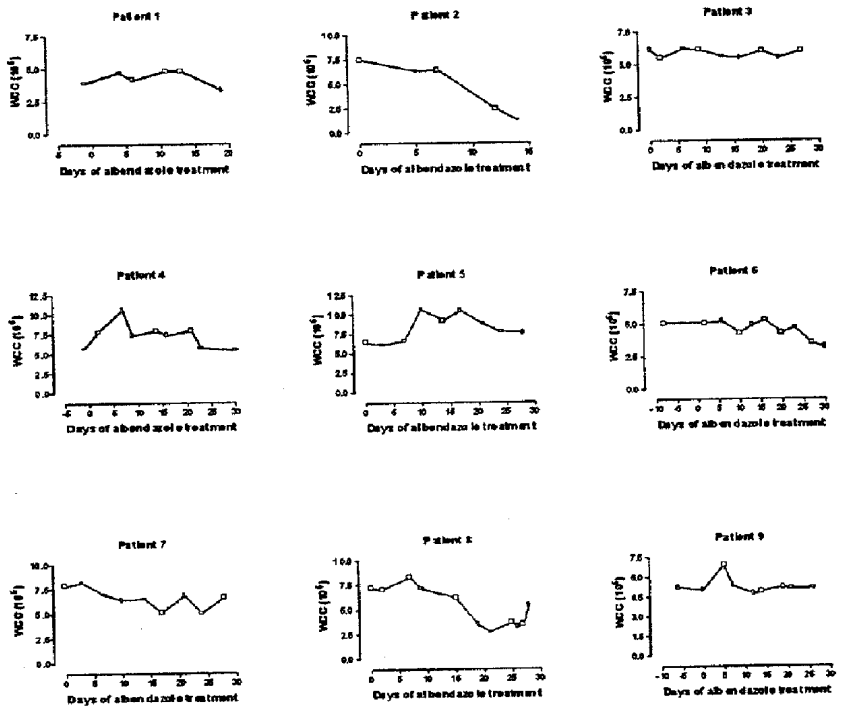

Patient 1
AFP (KU/L)
Days of albendazole treatment
Patient 2
CEA (μg/L)
Days of albendazole treatment
Patient 4
CEA (μg/L)
Days of albendazole treatment
Patient 5
CEA (μg/L)
Days of albendazole treatment
Patient 6
CEA (μg/L)
Days of albendazole treatment
Patient 7
CEA (μg/L)
Days of albendazole treatment
patient 8
CEA (μg/L)
Days of albendazole treatment

Figure 7:


Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
WBC (10⁹)
Days of albendazole treatment